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RESEARCH ARTICLE

The Effect of DBD-Non Thermal Plasma on Oxidative Stress and TAC in Patients With HCV Infection

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ABSTRACT

Non-thermal plasma (NTP) is a processing technique that is widely-spread in different biological and medical application which stimulates or inhibits cellular processes through releasing reactive oxygen and nitrogen species (ROS and RNS, respectively) in biological systems. In this study, it was investigated that the impact of NTP on oxidative stress and antioxidant capacity markers, as well as the effect of NTP on the copy number of Hepatitis C virus (HCV RNA). Dielectric barrier discharge (DBD) plasma was used to generate the NTP. The results showed that the exposure of HCV specimen to NTP causes oxidative stress, RNA damage, Protein denaturation, decrease total antioxidant capacity, and decreased copy numbers of HCV RNA in serum samples. In general, these results indicated that the NTP produced by DBD clearly decreases the level of total antioxidant capacity which reflects the total cooperative activities of antioxidants found in the serum.

Keywords: Dielectric barrier discharge, Hepatitis C virus, Non-thermal plasma, Oxidative stress, Total antioxidant capacity

Introduction

Non thermal plasma (NTP) is a state of partially or fully ionized gases contain various concentrations of atoms, ions, electrons, free electrical charges, which are generated at a low temperature by using argon gas.[1–3] At low pressure, NTP requires low power with ability to generate active chemical species (RNS) (NO_3^-, N^+_2, and NO) and reactive oxygen species (ROS) ($O_2^{\bullet-}$, $^1O_2$, O_3, $OH^\bullet$, and H_2O_2).[4] Dielectric Barrier Discharge (DBD) is the operating mechanism of the plasma discharge system. It produces high voltage (HV) pulses across the two electrodes after one of them becomes insulted.[5,6] NTP effectively controls the concentrations of intracellular ROS and RNS. NTP-generated nitric oxide species results from the elevation of intracellular nitric oxide concentration which finally leads to an increase in intracellular ROS.[7–9] Hepatitis C virus (HCV) is an RNA virus of Flaviviridae family virus which infect hepatic tissues causing moderate to severe liver injury.[10] HCV replicate inside cells causing cell necrosis through immune mediated cytolysis. Hepatic steatosis and oxidative stress (OS) with HCV infection increase ROS formation that finally cause protein denaturation, lipid peroxidation, and DNA damage to cells.[11,12] Hydroxyl radicals as ROS cause hepatic stellate cell activation, proliferation, and up regulate smooth muscle actin and collagen synthesis contributing to hepatic fibro genesis.[13] In addition,

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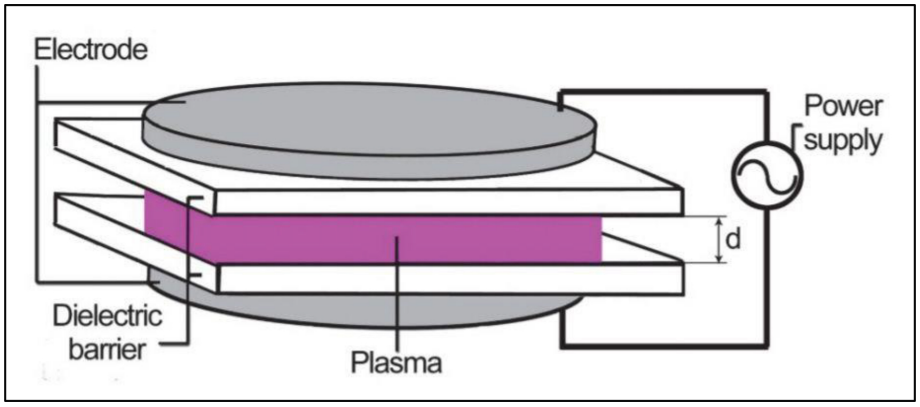

Fig. 1. Schematic diagram of DBD system.

many ROS are produced intrinsically by means of immunological processes in phagocytic cells or during regular biological activities in cells, including mitochondrial respiration and several enzymatic reactions like xanthine oxidase, among others.[14] The equilibrium between an organism's antioxidant responses and oxidizing agents is reflected in its antioxidant capacity.[15] Once extremely oxidative species surpass antioxidant thresholds, OS arises. Because free radicals are unstable and extremely reactive, they may damage biological components like DNA, proteins, and lipids and impair cellular activities if they are not eliminated. For this reason, OS is linked to the pathogenesis of serious chronic disorders like the hepatitis C virus.[16]

The goal of this study is to inactivation a hepatitis C virus by creating a balance between oxidative stress and antioxidants, thus; reducing the percentage of damage that occurs in body tissues through treatment with non-thermal plasma.

Materials and methods

Blood samples

In this study, participants were recruited from January 2023 to August 2023 at the Gastroenterology and Hepatology Center in Baghdad Medical City (Baghdad, Iraq) and Baqubah Teaching Hospital in Diyala province including patients with HCV. The diagnosis of HCV was made according to the clinical stages of each patient. The diagnosis of active HCV infection was based on test results over 3 months, in a study of 150 human subjects that included sixty HCV patients before and after NTP treatment and ninety healthy individuals as controls. These individuals were selected on the basis of no clinical or laboratory evidence of liver disease.

Specimen collection

Approximately 10 mL of fresh vinous blood was collected from healthy and patient volunteers. Samples were left for 30 minutes to clot at 25°C in gel tubes followed by 15 minutes centrifugation (1500 xg), then serum was collected and stored at –20°C for molecular investigations.

Dielectric barrier discharge

The dielectric barrier discharge (DBD) devise was designed and manufactured in our lab, it consists of two parts: the first one is high power supply to feed the probe with high voltage (up to 25 kV), and the second part is the floating electrode that consists of stainless-steel rod with diameter 2 mm and length (50 mm). The rod was covered by Pyrex glass tube with thickness (1 mm). One of the ends of tube was closed and the other was opened to connect the stainless-steel rod by cable to power supply. The open tube and cable were fixed inside PVC pipe acts as a handle which was attached vertically to movable stage, and blood serum was putted on the flat movable stage under the probe of the NTP. When the probe of NTP was approached to the blood serum, a distance about 1 mm and the power supply was switched on the NTP beginning to generate between the probe and the surface of the blood serum. The schematic diagram of DBD system is showed in Fig. 1.

Non-thermal plasma treatment

The HCV serum samples were exposed to NTP generated by DBD. 2 mL of HCV infected serum was loaded in a 24-well plate, NTP was applied at 1 mm distance from the solution surface within 240 seconds time duration. After NTP treatment, all the serum

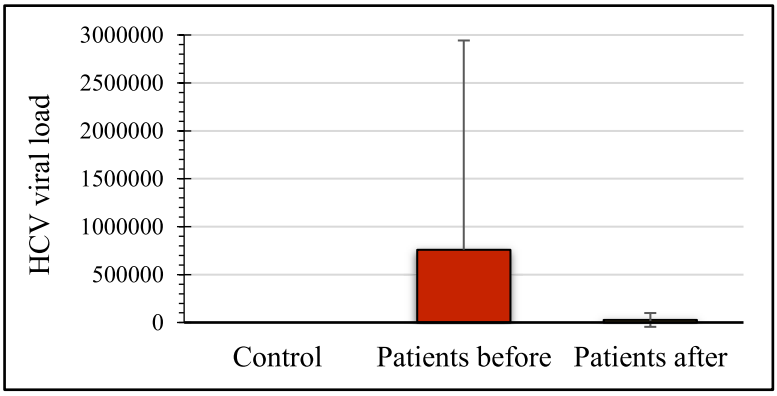

Fig. 2. HCV RNA results for hepatitis C patients before and after exposure to non-thermal plasma.

remained as liquid phase with no obvious changes in temperature. Control and NTP treated serum were transferred to an enzyme-linked immunosorbent assay (ELISA) plate for ROS and NO levels. Total antioxidant capacity (TAC) levels were determined using spectrophotometric technique, in addition; automated GeneXpert System was used to quantify non-NTP treated serum Hepatitis C Virus (HCV) RNA.

Gene expression quantitative method

Real-time quantitative polymerase chain reaction (RT-PCR) technology was used for viral load (VL) quantification of HCV Serum samples. Following manufacture instructions for RNS ki; 1.5 mL of serum was added to the cartridges which then were loaded inside the GeneXpert machine that is fully automated system combining nucleic acid amplification, specimen purification, and real-time reverse transcriptase PCR for the identification of a targeted gene in either simple or complicated specimens. This system uses a single-use disposable GeneXpert cartridges.

Two internal controls, which were the High and Low Internal Quantitative Standards (IQS-H/IQS-L) for recovered and inhibition activity tracking of the RT-PCR reactions, were subjected to the Xpert HCV VL cartridge for HCV RNA quantification in samples. The GeneXpert Instrument System automatically provided an interpretation of the results. Concerning the geneXpert system's limitation on analyzing serum and plasma materials for the world health organization reference material, it reports 3.20 IU/mL for continuous spectrum analysis (10 to 108 IU/mL) for HCV. [17]

Oxidative stress measurements

The kits of ROS and NO were purchased from Sun long Biotech Co., LTD, (China) were used. The test technique stated that each assay was performed using the equipment (Reader and Washer, HUMAN, Germany) and standardized using its calibration curve in accordance with the producer's guidelines. While, a colorimetric method using spectrophotometry technique was used to determine serum total antioxidant capacity in the samples following Earl et al. method. [18,19]

Statistical analysis

Results were analyzed statistically using social sciences (SPSS) Statistics version 26 program. All the study results are shown to be mean ± standard division (SD). Receiver operating characteristic (ROC) curve was dependent to evaluate the area under the curve (AUC), and the best cut off point of the studied markers, sensitivity, and specificity. Statistical graphs were generated using Graph Pad Prism version (9.3). Probability values of ($p < 0.05$) was considered significantly different.

Results and discussion

Serum was obtained from 150 human subjects including 60 HCV patients and 90 healthy controls aged (20 to 60 years). Using demographic characteristics interpretations, there were no significant differences ($P > 0.05$) between the two groups in regard to age and body mass index.

The effect of NTP on HCV RNA was investigated by Cepheid Xpert HCV Viral Load assay. According to results, there was significant ($p < 0.05$) improvement of HCV RNA degradation. Fig. 2 and Table 1 show that the NTP treatment with 240 exposure decreased copy numbers of HCV RNA.

As shown in Table 1 and Fig. 3, NTP exposure for 240s leads to a decrease in TAC levels. These results indicate that NTP treatment has a significant effect on TAC that will be released from its binding to HCV

Table 1. Control, HCV Viral Load, ROS, NO and TAC results for hepatitis C patients before and after exposure to non-thermal plasma.

Parameters	Control	Patients before	Patients after
HCV RNA (IU/mL)	–	758618.77 ± 2184197.47	26834.75 ± 72145.62
ROS (pg/mL)	224.57 ± 62.32	278.76 ± 116.51	318.41 ± 160.88
NO (ng/mL)	24.93 ± 9.41	58.99 ± 25.14	71.71 ± 28.65
TAC (μmol vit. C Eq/L)	53.81 ± 9.92	47.52 ± 13.33	41.96 ± 11.85

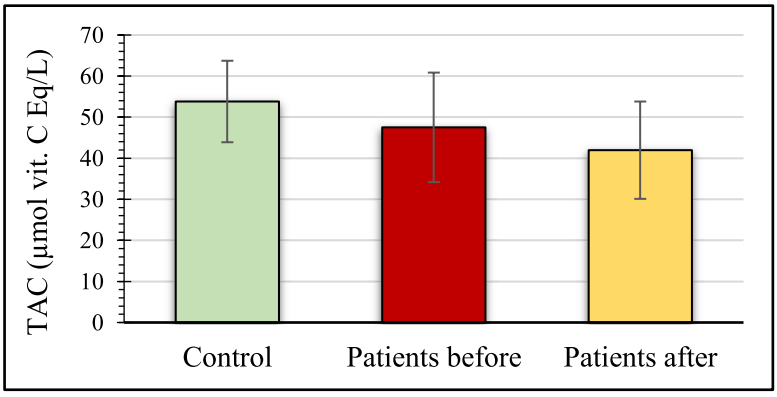

Fig. 3. TAC results for hepatitis C patients before and after exposure to non-thermal plasma.

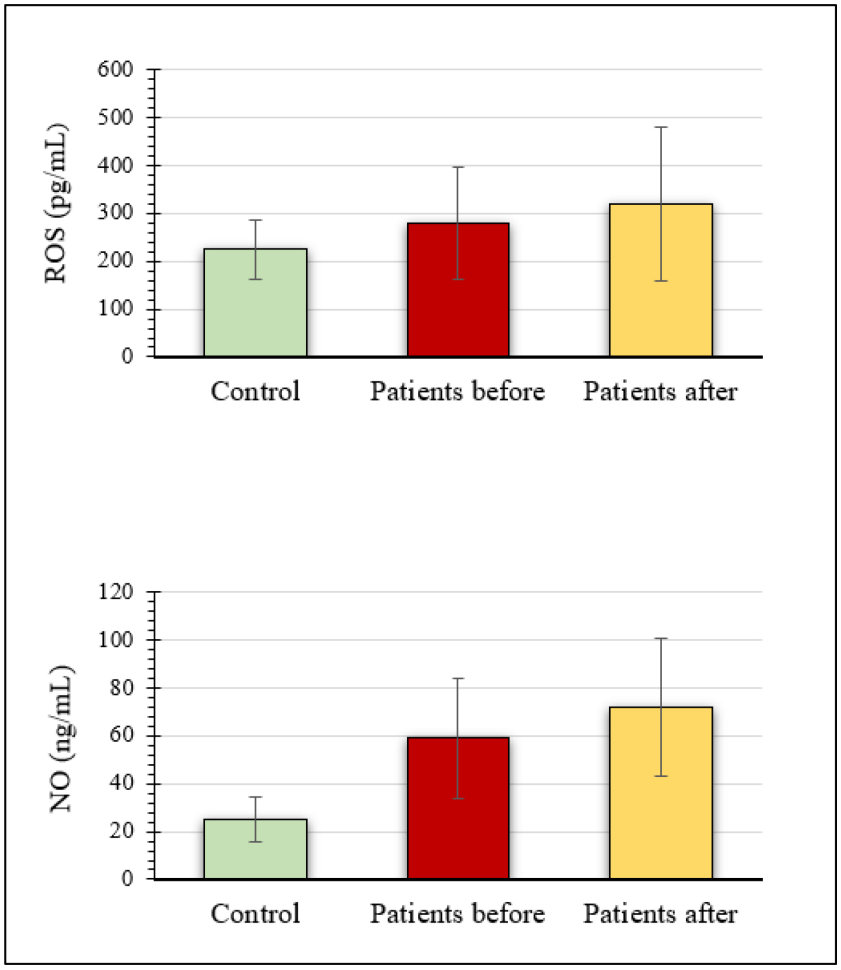

Fig. 4. ROS and NO results for hepatitis C patients before and after exposure to non-thermal plasma.

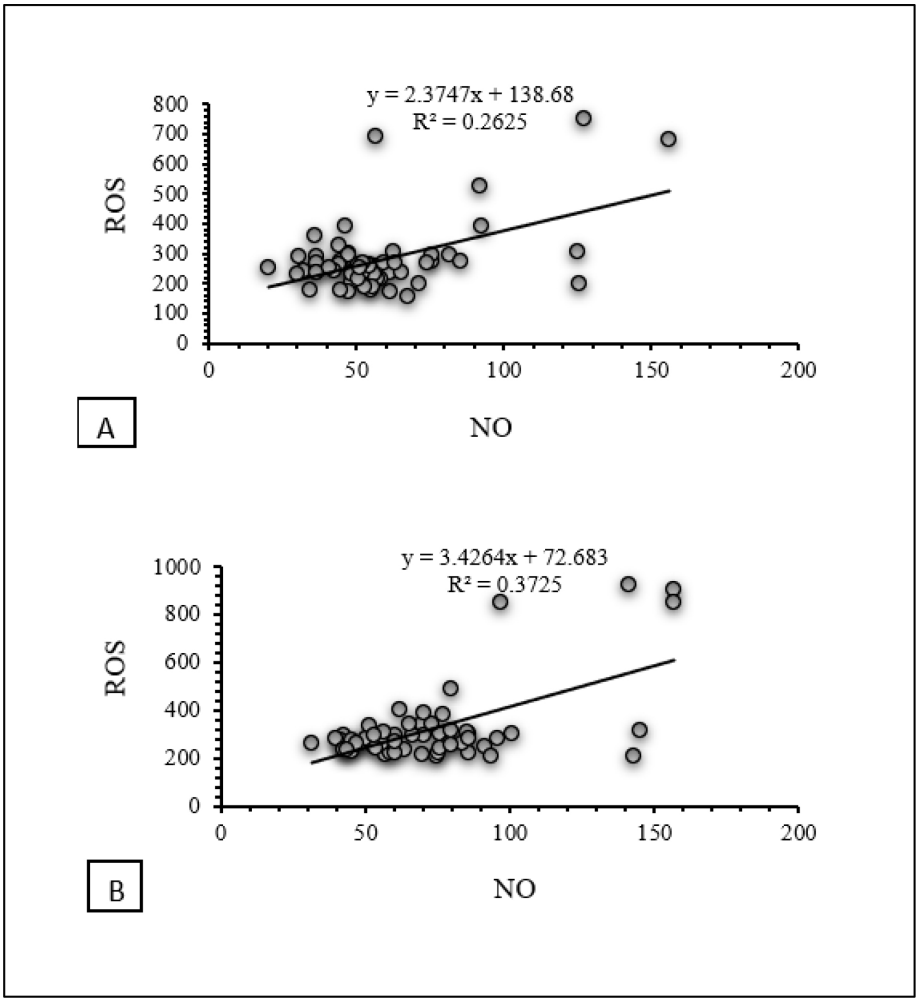

Fig. 5. Pearson correlation analysis between ROS and NO results for hepatitis C patients (HCV) before and after exposure to non-thermal plasma, (A) before exposure to NTP, (B) after exposure to NTP.

proteins, thus acts as a stimulating factor. Detoxification enzyme was compared with other biomarkers of oxidative stress in the same sample. There was no significant correlation between TAC, ROS and NO levels in HCV patients before and after exposure to NTP.

ROS and NO indicators, such as biomarker of oxidative stress, are shown in Table 1 and Fig. 4. They indicate that the level of ROS and NO increases after NTP application, and these results indicate that NTP treatment plays an important role increasing oxidative stress. There was a positive significant correlation between ROS and NO levels in HCV patients before and after plasma are shown in Fig. 5.

Receiver operating characteristic (ROC) curves in logistic regression is a useful approach to predicting whether an observation is true or false. The ROC curve was employed for the prediction of liver injury using ROS, NO and TAC for HCV patients. These results of ROC analysis indicated that all of these parameter's ROS, NO and TAC possessed a sensitivity of 71.7%, 91.7% and 64.4% a specificity of 68.9%, 82.2% and 55% ($p < 0.001$, < 0.001 and < 0.012) respectively for the detection of injury in patients with HCV infection. Thus, the combination of these parameters ROS, NO and TAC can be used as a predictor of liver injury degree in HCV infection as shown in Table 2, Fig. 6.

Table 2. Receiver operating characteristic (ROC) analysis ROS, NO and TAC results for hepatitis C patients.

Parameter	AUC	SE	p-value
ROS	0.672	0.045	<0.001
NO	0.954	0.017	<0.001
TAC	0.622	0.047	0.012

The development of new antiviral strategies for HCV-infected patients is crucial. Specifically, these strategies should target RNA directly.[20] In this research we constructed DBD plasma system to

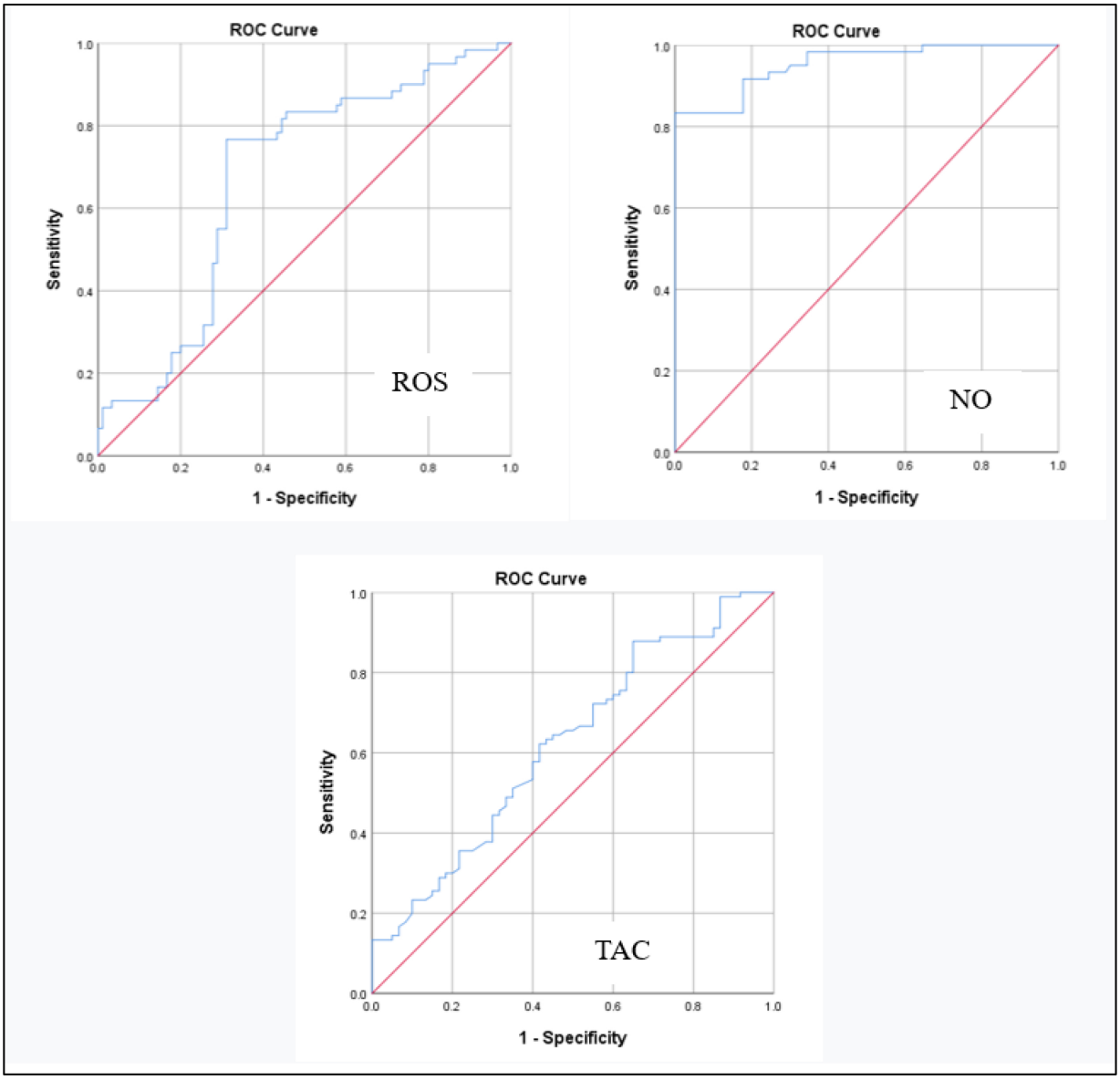

Fig. 6. Receiver operating characteristic (ROC) analysis ROS, NO and TAC results for hepatitis C patients.

generate NTP for the therapy of hepatitis C virus.[21] This technology generates wide spectrum of reactive oxygen and nitrogen species (ROS and RNS, respectively), in which it is an important factor that reduces the HCV activity. An increase in ROS and NO production can cause cell cycle arrest or induce apoptosis through damages to biological macromolecules structures such as DNA, proteins denaturation, lipids peroxidation.[22] The expected mechanism for inactivation of the virus is through molecules and ions generated by DBD-NTP, such as such as ROS ($O_2^{\bullet-}$, $^1O_2$, O_3, $OH^{\bullet}$, and H_2O_2) and (RNS) (NO_3^-, $N^+{}_2$, and NO) which are short-lived and work to destroy the cell wall of the virus, which leads to the destruction of lipids and proteins and damage to the RNA.[6] These factors, such RONS can affect growth factors release and intracellular ROS levels, thus they regulate cell activities. NTP produces ROS, RNS, NO, electrons, and ions generated from the DBD-NTP which cross the virus envelope membrane barrier reaching inside the virus through pores result from lipid peroxidation of the membrane.[23] In consideration to age, diffusion rate, and membrane barriers crossing, RON's precise interaction with different tissue components is not fully understood. Generated RONS or intracellular RONS stimulation as a result of DBD-NTP therapy was proposed for cell activity regulation. ROS production by DBD-NTP therapy stimulates the fibroblast

growth factor-2 [FGF-2] release timely; in addition, it promotes endothelial cell proliferation. The usage of NTP at low intensity with short period of time do not have any significant effect on the activity of normal cells, however it can induce virus inactivation. DBD-NTP effects on cell activity due to reactive oxygen species levels production under different conditions. It has been found that the generation of ROS and NO occurs during infection with the HCV and inflammatory procedures, and during cell ischemia, as well as a decrease in the TAC. This leads us to an extensive study in this field because it has positive and negative effects at the same time. It works to destroy the HCV, but in return it causes to increase oxidative stress and decrease of the total antioxidant capacity, through which we suggest giving external antioxidants to create a balance between oxidative stress and antioxidant. ROS has been obtained from non-thermal plasma and used to biological purposes. Further investigation is required in this field, encompassing studies concerning ROS processes within cells and the application of reactive species in redox medicine. These investigations will contribute to the development of effective treatment strategies for a range of illnesses.

Conclusion

In conclusion, the result of this study revealed that DBD- NTP inactivation of the virus decreased copy numbers of HCV RNA number of the virus, but in return increase the level of oxidative stress and highlights the pivotal role that TAC plays in regulating cellular defenses against oxidative stress. The results so showed that DBD-NTP treatment has a positive effect on oxidative stress. The use of DBD- NTP leads to the stimulation of stress-sensing cell reactions along the low levels of antioxidant defense system enzymes.

Acknowledgment

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Diyala.

Authors' contribution statement

Thermotical concept (S. K. A., S. N. A., H. H. M., M. R. M.), experimental design (S. K. A., S. N. A., H. H. M., M. R. M.), experiments working (S. K. A., S. N. A., H. H. M., M. R. M.), statistical process (S. K. A., S. N. A.), manuscript writing (S. K. A., S. N. A., H. H. M., M. R. M.), manuscript revise (S. K. A., S. N. A., H. H. M., M. R. M.).

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تأثير البلازما غير الحرارية (DBD) على الإجهاد التأكسدي ومضادات الاكسدة الكلية في المرضى المصابين بعدوى فيروس التهاب الكبد الوبائي نوع سي

سليمه كريم احمد[1] ، سلوى حميد ناصر الربيعي[2] ، حامد حافظ مربط[3] ، مازن رزوقي محمد[4]

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الخلاصة

البلازما غير الحرارية (NTP) هي تقنية معالجة منتشرة على نطاق واسع في مختلف التطبيقات البايولوجية والطبية. من خلال إطلاق أنواع الأوكسجين والنيتروجين التفاعلية (ROS وRNS، على التوالي)، فإنه يسمح بتحفيز أو تثبيط العمليات الخلوية في النظم البيولوجية. في هذه الدراسة، تمت دراسة تأثير NTP على الإجهاد التأكسدي وعلى القدرة الكلية لمضادات الأكسدة وكذلك تأثير NTP على عدد نسخ فيروس التهاب الكبد الوبائي نوع سي (HCV RNA). تم استخدام بلازما تفريغ الحاجز العازل (DBD) لتوليد البلازما غير الحرارية، حيث أظهرت نتائج التقييم أن تعرض عينة فيروس التهاب الكبد الوبائي سي (HCV) لـ NTP يسبب الإجهاد التأكسدي، وتلف الحامض النووي الريبوزي (RNA)، وتمسخ البروتين، وانخفاض في مستوى القدرة الكلية لمضادات الأكسدة وانخفاض أعداد نسخ HCV RNA في عينات المصل. بشكل عام أشارت هذه النتائج إلى أن NTP الذي ينتجه DBD يقلل بشكل واضح من مستوى القدرة الكلية لمضادات الأكسدة مما يعكس إجمالي الأنشطة التعاونية لمضادات الأكسدة الموجودة في المصل.

الكلمات المفتاحية: البلازما غير الحرارية، تفريغ الحاجز العازل، التهاب الكبد الوبائي سي، الاجهاد التأكسدي، القدرة الكلية لمضادات الاكسدة.